Neural disease: *Drosophila* degenerates for a good cause

Mousumi Mutsuddi and John R. Nambu

Human neurodegenerative disorders are typified by late onset cell loss in specific brain regions and stereotypic neuroanatomical and behavioral aberrations. Recent studies suggest that molecular genetic approaches in *Drosophila* may shed important new light on conserved mechanisms underlying such disorders.

Address: Biology Department, Morrill Science Center, University of Massachusetts, Amherst, Massachusetts 01003, USA.

Current Biology 1998, 8:R809–R811
http://biomednet.com/elecref/09609822008R0809

The most notorious human neurodegenerative disorders, such as Alzheimer's disease, Huntington's disease, Parkinson's disease and amyotrophic lateral sclerosis, typically have a late onset, causing premature death after a period during which the symptoms inexorably worsen. Afflicted individuals may exhibit behavioral deficits, such as ataxia, seizures and tremors, as well as cognitive impairments involving memory loss or dementia. These disorders are associated with moderate to severe cell loss and anatomical disruptions of specific brain regions. Genes that might underlie inherited forms of some of the neurodegenerative diseases have been identified, but the mechanisms by which disruptions in these genes result in specific disease pathologies are not yet clear [1].

Given the inherent limitations on genetic analysis in humans, it would clearly be helpful to have relevant models of human neurodegenerative diseases in genetically tractable species. Recent studies give strong indications that the fruit fly *Drosophila* will be a valuable experimental subject for studying conserved mechanisms underlying human neurodegenerative disorders (see [2] for example). We shall discuss these developments and the complementary experimental approaches that are being taken to studying neurodegeneration in *Drosophila*.

Drosophila neurodegeneration mutants and genes

A number of *Drosophila* mutants have been identified that exhibit symptoms reminiscent of human neurodegenerative disorders, including some or all of reduced adult longevity, aberrant behavior and stereotypic brain lesions. Some examples of these are listed in Table 1. In several instances, specific phenotypic similarities to human neurodegenerative disorders have been noted. For example, peripheral nerve hypomyelination or hypermyelination by Schwann cells is associated with several human neuropathies [3], and similar defects in glial wrapping of neuron cell bodies and axons are found in the *Drosophila* mutants *drop dead* [4] and *swiss cheese* [5].

Wild-type adult fruit flies generally live for 2–3 months, but *drop dead* mutants typically die within their first week of adulthood. Although newly emerged *drop dead* flies appear normal, they soon exhibit decreased motor activity, and their brain glia have stunted cytoplasmic processes that fail to enwrap neighboring neurons properly. Adult *drop dead* mutants also show accelerated age-dependent changes in gene expression patterns [6], implying that aging processes may be disrupted. The *drop dead* gene encodes a novel transmembrane protein expressed in brain tracheole cells (S. Benzer, personal communication), suggesting that the brain defects in *drop dead* mutants may arise at least in part from hypoxia.

In *swiss cheese* mutant flies, large vacuoles form in the brain and widespread neuronal and glial cell death is detected. Glial processes form abnormal, multi-layered wrappings around neurons and axons in the mutant brains. The *swiss cheese* gene is expressed by neurons in the brain cortex and encodes a membrane protein related to the human protein known as neuropathy target esterase [7]. Chemical inhibition of neuropathy target esterase has been shown to result in a delayed degeneration of long axons in the spinal cord and peripheral nerves. The Swiss Cheese protein also contains a region of homology to the cAMP-binding regulatory subunit of protein kinase A [5,7], which is thought to be an important component of intracellular signaling pathways in vertebrate neurons and glia.

Drosophila spongecake mutants show temperature- and age-dependent formation of large vacuoles and degeneration of neuronal axons in the optic lobes [8]. The axon terminals swell and neighboring axons often coalesce to form vacuolar structures similar in appearance to those found in brains of humans with Creutzfeld–Jakob disease. Mutations in the *eggroll* gene result in widespread degeneration in the optic lobe and central brain, followed by retinal degeneration [8]. In *eggroll* mutant brains, neurons and glia contain multi-laminar structures similar to those seen in humans with Tay–Sachs disease. These structures are detected in the brains, not only of adults, but also of third instar larvae, indicating that cellular defects begin to accumulate well before symptoms are evident.

A number of human and fly neurodegeneration disorders result in very specific behavioral aberrations. The *Drosophila pirouette* mutant exhibits auditory deficits and age-dependent circling behaviors that progress in severity

Table 1

***Drosophila* neurodegeneration mutants and genes.**

Gene or mutation	Mutant phenotype	Human homolog
drop dead [4]	Glial hypo-wrapping	None known
swiss cheese [5,7]	Brain vacuoles, glial hyperventilation	Neuropathy target esterase
spongecake [8]	Axon degeneration and fusions	ND
eggroll [8]	Neuronal and glial degeneration, multi-laminar structures	ND
Vacuolar medulla [18]	Optic lobe degeneration, optomotor response defects	ND
pirouette [9]	Circling behavior, brain degeneration	ND
beta-amyloid protein precursor-like [11]	Locomotor defects	β-amyloid precursor
Superoxide dismutase [12]	Retinal degeneration	Cu^{2+}/Zn^{2+} superoxide dismutase
ripped pocket / pick pocket [19]	ND	Degenerin sodium channel subunit
Presenilin [20,21]	ND	Presenilins
retinal degeneration C [2]	Light-induced degeneration	Serine/threonine phosphatase
neither inactivation nor action potential E [2]	Retinal degeneration	Rhodopsin

ND, not yet determined.

from an abrupt 'about face' turning behavior to turning in increasingly tighter circles [9]. These flies also exhibit massive, often asymmetric degeneration of brain tissues. Similar circling behaviors are exhibited by several mouse neurological mutants, as well as by humans with a disorder known as hemi-parkinsonism [10].

Gene-specific lesions in *Drosophila* can thus induce neural defects with many of the hallmarks of human neurodegenerative disorders. One crucial question is whether defects in homologous genes can cause similar neurodegeneration in flies and humans. A number of *Drosophila* homologs have been identified of human genes that have been linked to neurodegenerative disease (Table 1). The *Drosophila* gene *beta-amyloid protein precursor-like* (*Appl*) [11], for example, encodes a homolog of the human β-amyloid precursor protein (APP), which gives rise to β-amyloid, a major component of the plaques in the brains of Alzheimer's disease sufferers. In mice, *APP* mutations result in reduced locomotor activity as well as glial degeneration. In *Drosophila* the Appl protein is exclusively expressed in differentiated neurons, and deletions of the *Appl* gene result in adult locomotor defects [11]. These behavioral defects can be partially rescued by ubiquitous expression of either fly *Appl* or human *APP*, indicating that these homologous genes are functionally conserved.

A *Drosophila* homolog [12] has also been identified of the human gene for the Cu^{2+}/Zn^{2+} superoxide dismutase. Mutations of this gene have been implicated in Lou Gehrig's disease, a familial form of amyotrophic lateral sclerosis that results in motor neuron degeneration and paralysis. Deletion mutations of the *Drosophila superoxide dismutase* gene cause a reduction in lifespan and hypersensitivity to oxygen stress, while at least one missense mutation of the gene results in degeneration of photoreceptor neurons and pigment cells throughout the retina.

Glutamine-repeat disease in *Drosophila*

Many dominantly-inherited human neurodegenerative diseases, including Huntington's disease, spinocerebellar ataxia and myotonic dystrophy, are caused by expansions of trinucleotide repeats coding for glutamine residues in a specific gene product [13,14]. Similar neurological phenotypes can be induced in transgenic mice 'models' expressing the corresponding proteins with expanded glutamine-repeat regions. The glutamine-repeat diseases share several distinctive properties. Firstly, the symptoms arise only when the repeat number exceeds a characteristic threshold for each gene. Secondly, affected lineages show genetic 'anticipation': repeat lengths increase in successive generations, resulting in increasing severity and earlier onset of symptoms. And thirdly, although most of these proteins are normally cytoplasmic and widely expressed, the disease-associated versions aggregate in nuclear inclusions present only in the affected cells.

Two important questions concerning neurodegenerative diseases of this type immediately arise. These are, firstly, what is the relationship between nuclear inclusion formation and disease pathogenesis? And secondly, are there common pathogenic mechanisms that underlie the various glutamine-repeat diseases? The answers to these questions are not known, but it has been suggested that the expanded poly-glutamine tracts might act as 'polar zippers' that can mediate aberrant protein–protein interactions, or as aberrant substrates for cross-linking by transglutaminases. Either property could lead to the mutant proteins promoting the formation of heterologous insoluble protein aggregates that may ultimately result in the titration and loss of activity of vital cellular proteins.

In *Drosophila*, there are a large number of proteins that contain poly-glutamine tracts and function in nervous system development [15]. Although there are as yet no known examples of triplet-repeat expansion in an endogenous *Drosophila* gene that cause a neurodegenerative phenotype, naturally-occurring polymorphisms in sequences

encoding poly-glutamine tracts have been observed [16]. And it was recently found that a human protein with an expanded glutamine repeat region can induce neurodegeneration in *Drosophila*. This was accomplished by targeted expression of a transgene coding for a mutant protein associated with Machado Joseph's syndrome [17], a common form of spinocerebellar ataxia. Expression of a normal version of the protein, MJDtr-Q27, with 27 glutamine repeats, had no phenotypic effects in any of several tissues examined. This form of the protein was consistently cytoplasmic, and no nuclear inclusion formation was seen. In contrast, expression of the mutant form MJDtr-Q78, with 78 glutamine repeats, led to reduced lifespan, age-dependent cell loss and nuclear inclusion formation. These effects showed apparent dosage-sensitivity and strong tissue specificity, suggesting that, as in humans, other, cell-specific factors are essential for pathogenesis.

MJDtr-Q78 had a dynamic subcellular localization in the transgenic flies that varied with age. During eye imaginal disc development, for example, the protein moved from cytoplasm to nucleus in differentiating photoreceptor neurons. This nuclear localization correlated with nuclear inclusion formation and subsequent cellular degeneration. Interestingly, nuclear inclusions were also detected in epithelial cells of the imaginal discs, where no degeneration was observed. Thus, at least in *Drosophila*, nuclear inclusion formation is not sufficient to cause degeneration.

An important goal of such studies will be to decipher the basis for the tissue-specific effects of expanded glutamine-repeat proteins. With *Drosophila*, it should be possible to identify other genes involved in disease pathogenesis by carrying out genetic screens for second-site modifiers of MJDtr-Q78-induced phenotypes. This may permit identification of genes encoding proteins that specifically inhibit or support nuclear inclusion formation, and that might directly or indirectly interact with MJDtr-Q78. This approach should strongly complement other methods used to identify tissue-specific factors that directly associate with expanded poly-glutamine tracts [13,14].

It will be of interest to see whether other human glutamine-repeat proteins induce distinct neurodegeneration phenotypes when their mutant forms are expressed in transgenic fruit flies. If so, perhaps *Drosophila* can be used to help identify both common and gene-specific mechanisms underlying the various glutamine-repeat diseases. Other issues of interest are whether the repeats are genetically unstable and undergo expansion in *Drosophila*, and whether this results in 'anticipation'. There may exist species-specific aspects of genome organization or DNA metabolism that affect the accurate maintenance of triplet repeats, and perhaps distinct thresholds of repeat numbers required to induce neurodegeneration. At any rate, repeat expansion is not a requisite for effectively modeling glutamine-repeat disease, and analyzing any mechanistic differences should also be highly informative.

Acknowledgements

The authors are indebted to Larry Schwartz and Ashim Mukherjee for helpful comments on the manuscript, and to Seymour Benzer for sharing unpublished results.

References

1. Roses AD: From genes to mechanisms to therapies: lessons to be learned from neurological disorders. *Nat Med* 1996, 2:267-269.
2. Davidson FF, Steller H: Blocking apoptosis prevents blindness in ***Drosophila retinal degeneration*** mutants. *Nature* 1998, 391:587-591.
3. Griffiths IR: Myelin mutants: model systems for the study of normal and abnormal myelination. *BioEssays* 18:789-797.
4. Buchanan RL, Benzer S: Defective glia in the ***Drosophila*** brain degeneration mutant ***drop-dead***. *Neuron* 1993, 10:839-850.
5. Kretzschmar D, Hasan G, Sharma S, Heisenberg M, Benzer S: The ***swiss cheese*** mutant causes glial hyperwrapping and brain degeneration in ***Drosophila***. *J Neurosci* 1997, 17:7425-7432.
6. Rogina B, Benzer S, Helfand SL: ***Drosophila drop-dead*** mutations accelerate the time course of age-related markers. *Proc Natl Acad Sci USA* 1997, 94:6303-6306.
7. Lush MJ, Li Y, Read DJ, Willis AC, Glynn P: Neuropathy target esterase and a homologous ***Drosophila*** neurodegeneration-associated mutant protein contain a novel domain conserved from bacteria to man. *Biochem J* 1998, 332:1-4.
8. Min K-T, Benzer S: ***Spongecake*** and ***eggroll***: two hereditary disease in ***Drosophila*** resemble patterns of human brain degeneration. *Curr Biol* 1997, 7:885-888.
9. Eberl DF, Duyk GM, Perrimon N: A genetic screen for mutations that disrupt an auditory response in ***Drosophila melanogaster***. *Proc Natl Acad Sci USA* 1997, 94:14837-14842.
10. Bracha HS, Shults C, Glick SD, Kleinman JE: Spontaneous asymmetric circling behavior in hemi-parkinsonism; a human equivalent of the lesioned-circling rodent behavior. *Life Sci* 1987, 40:1127-1130.
11. Luo L, Tully T, White K: Human amyloid precursor protein ameliorates behavioral deficit of flies deleted for ***Appl*** gene. *Neuron* 1992, 9:595-605.
12. Phillips JP, Tainer JA, Getzoff ED, Boulianne GL, Kirby K, Hilliker AJ: Subunit-destabilizing mutations in ***Drosophila*** copper/zinc superoxide dismutase: neuropathology and a model of dimer dysequilibrium. *Proc Natl Acad Sci USA* 1995, 92:8574-8578.
13. Perutz MF: Glutamine repeats and inherited neurodegenerative diseases: molecular aspects. *Curr Opin Struct Biol* 1996, 6:848-858.
14. Ross CA: Intranuclear neuronal inclusions: a common pathogenic mechanism for glutamine-repeat neurodegenerative diseases? *Neuron* 1997, 19:1147-1150.
15. Karlin S, Burge C: Trinucleotide repeats and long homopeptides in genes and proteins associated with nervous system disease and development. *Proc Natl Acad Sci USA* 1996, 93:1560-1565.
16. Michalakis Y, Veuille M: Length variation of CAG/CAA trinucleotide repeats in natural populations of ***Drosophila melanogaster*** and its relation to the recombination rate. *Genetics* 1996, 143:1713-1725.
17. Warrick JM, Paulson HL, Gray-Board GL, Bui QT, Fischbeck KH, Pittman RN, Bonini NM: Expanded polyglutamine protein forms nuclear inclusions and causes neural degeneration in ***Drosophila***. *Cell* 1998, 93:939-949.
18. Coombe PE, Heisenberg M: The structural brain mutant ***Vacuolar medulla*** of ***Drosophila melanogaster*** with specific behavioral defects and cell degeneration in the adult. *J Neurogenet* 1986, 3:135-158.
19. Adams CM, Anderson MG, Motto DG, Price MP, Johson WA, Welsh MJ: Ripped pocket and pickpocket, novel ***Drosophila*** DEG ENaC subunits expressed in early development and in mechanosensory neurons. *J Cell Biol* 1998, 140:143-152.
20. Boulianne GL, Livne-Bar I, Humphreys JM, Liang Y, Lin C, Rogaev E, St George-Hyslop P: Cloning and characterization of the ***Drosophila*** presenilin homologue. *Neuroreport* 1997, 8:1025-1029.
21. Hong CS, Koo EH: Isolation and characterization of ***Drosophila*** presenilin homolog. *Neuroreport* 1997, 8:665-668.